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# Pb and Zn contamination as rate-regulation agents on decomposition of two emergent macrophytes: a systemic approach

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## Abstract

Modeling the carbon cycling in aquatic environments is relevant for predicting the detritus processing capacity in metal-affected environments. Considering the potential toxic effect of Zn and Pb on microbial metabolism, we hypothesize that the presence of these metals decreases decomposition rates of macrophytes detritus. We examined the anaerobic decomposition of *Hedychium coronarium* and *Urochloa subquadripara* in presence of Zn and Pb at 15 and 25°C. The mass decay was parameterized by first-order kinetic model. The higher concentration of Zn promoted reduction on mineralization of particulate organic carbon (POC) in the *U. subquadripara* and *H. coronarium* detritus. The $Q_{10}$ (*H. coronarium*: 0.95 to 1.75 and *U. subquadripara*: 1.26 to 1.59) showed a positive effect of temperature increasing during macrophytes anaerobic degradation. Considering the metal type and concentration, the high Zn concentration acted as a driving force over POC decay and consequently affecting the yields in the plant detritus mineralization processes since the decrease in POC degradation was effective due to adsorption of metals in this detritus fraction. The use of mathematical models was suitable tool that allows predicting carbon turnover in an ecosystem submitted to an episode of metal contamination.

Keywords: Metals; Degradation; Palustrine plants; Kinetic model; Tropical savanna reservoir.

## INTRODUCTION

Metals are a common environmental contaminant, usually found in soil, water, sediments and air, resulting from natural (e.g. crustal material) or antrophogenic activities, as urbanization and industrialization. There are many studies about the metal effects on aquatic populations, communities, and food web (Magalhães *et al.*, 2015; Nweke *et al.*, 2018) but just few studies have focused the metal effects on decomposition that is responsible by elements cycling. Considering that metals are toxic, persistent and not degradable, these properties led to concern over the short and long-term actions of metals in the aquatic ecosystem. Regarding to microbial metabolism, the metals considered to be of concern are arsenic, chromium, cadmium, copper, lead, mercury, nickel, selenium and zinc (Bitton, 2002), and these elements are often associated with industrial activities (Berg & McClaugherty, 2014).

Tropical shallow aquatic environments (e.g. wetlands, oxbow lakes) are one the most productive ecosystem and are, eventually, exposed to metal contamination due to anthropic action (Valitutto *et al.*, 2006). In these aquatic systems, macrophytes and algae are the main source of autochthonous organic matter (Bertilsson & Jones Jr., 2003). After death, the plant detritus sustains decomposition, acting as an important energy source for aquatic heterotrophy organism, playing an essential role in the biogeochemical cycles of C, N and P. The extrinsic factors as pH and water trophic state (chemical drive functions) and temperature (physical drive function) conditioned organic matter decay (Harrison, 1989). Also intrinsic factors as detritus C:N:P stoichiometry (Scott *et al.*, 2013) and litter quality (Guendehou *et al.*, 2014) associated with heterotrophic enzymatic ability of microbial community affect the elements turnover. Depending on the metal concentration in aquatic environments, they can be

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*Corresponding author: M. B. Cunha-Santino; e-mail: cunha_santino@ufscar.br considered an extrinsic factor that acting as micronutrient requirement to the microbial community (Overmann, 2006), and consequently, increasing the turnover rates of elements due to decomposition, or playing a toxic role on microbial metabolism that mediated degradation. The metal effect can also be identified in the decay process by enhanced or decreasing the decomposition rates (Pradhan *et al.*, 2011).

Liquid tailings are on type of waste produced from mineral extraction and usually present high amounts of metals. The high concentrations of metals in the aquatic ecosystem is a serious environmental issue once the presence of these elements disrupts the ecosystem services as decomposition due to its toxic feature and non-degradative nature. Considering that metals affect the heterotrophic metabolism of macro (Chiba *et al.*, 2011) and microorganisms (Zlatković, 2017) responsible for decomposition it leads to loss in the processing ability of organisms responsible for carbon cycling with a consequent disturbance on ecosystem functioning. Metal contamination of drainage areas at mining and industrial sites involves complex interactions on organic matter cycling. Lead and zinc are typical constituents present in battery industries generating non-treated effluents (Mahvi *et al.*, 2014) and often, the concentrations of these metals exceed the maximum values recommended by the Brazilian legislation (Conama Resolution nº 357/2005). Metals, as Zn and Pb, are among those with higher concentrations in mining tailings (Kossoff *et al.*, 2014). In Brazil, the analysis of mine tailing that reached the Doce River basin in November 2015, the largest Brazilian socio-environmental disaster, presented high metals (Fe, Mn, Cr, Zn, Ni, Cu, Pb and Co) contents; the mean values of Zn and Pb: 62.4 ± 28.4 mg kg$^{-1}$ and 20.2 ± 4.6 mg kg$^{-1}$, respectively (Queiroz *et al.*, 2018). Impacts from abandoned mines, dam collapse or industrial activities on water quality require an understanding of the interaction of metal contaminant in the carbon cycling.

Toxicity depends on several factors such as geochemical behavior of contaminant that is directly dependent on environmental conditions, the dose, the duration (i.e. time of exposure) and physiology of the target organism (Sparling, 2017). Considering the potential toxic effect of Zn and Pb concentrations tested, we hypothesize that the presence of these metals decreases macrophytes decomposition rates in aquatic environments, affecting the carbon turnover. The aims of this study were to parametrize the effect of Zn and Pb presence on the anaerobic decomposition of *Hedychium coronarium* and *Urochloa subquadripara* at two temperatures. We used mathematical modeling to establish the yields and rates from decomposition in the presence of metals in order to predict implications for long-term organic matter decay on an eventual scenario of metal-affected environment.

## MATERIAL AND METHODS

### *Study site description and field procedure*

*Hedychium coronarium* J. Koenig and *Urochloa subquadripara* (Trin.) R. D. Webster are considered invasive palustrine plants in Brazilian savanna (Zenni & Ziller, 2011) usually located on the margin of streams, ponds and reservoirs. Whole adult individuals (leaf, culm, root and ground rhizome) of *H. coronarium* and *U. subquadripara* were manually collected at the littoral zone of the Fazzari Reservoir (23K 201.738 UTM 7.567.523) and the Espraiado Reservoir (23K 204.575 UTM 7.563.434), respectively. These aquatic environments were located within the Savanna biome, São Carlos municipal district, São Paulo State, Brazil. Subsurface (Z = 15 cm) water and sediment samples (ca. 500 g in 500 ml of water) were collected with using an Ekman-Birge bottom grab sampler and a Van-Dorn bottle, respectively, in the littoral zone of each location. The sediments were used as a source of indigenous microbial inoculums in the decomposition assays. The inoculants were set up with 100 ml of fresh sediment and 1 L of water from the collection site. In the laboratory, the water samples were immediately filtered (cellulose acetate membrane, 0.45 µm pore-size). Before setup the incubations, the samples of *H. coronarium* and *U. subquadripara* were washed with deionized water for removing adhered coarse material, oven-dried under 40°C until constant mass, and fragmented in pieces varying from 5 to 9 cm.

### *Metal concentration of water samples, macrophytes and metal sorption assay*

The Zn and Pb were analyzed in water samples from the Fazzari and the Espraiado Reservoirs and in the tissues (DW) of *H. coronarium* and *U. subquadripara* and used as background value in the sorption assays. Immediately after water sampling, an aliquot was acidified with concentrated $HNO_3$ and filtered with 0.45 µm pore-size, cellulose acetate membranes. The quantification of metals was conducted after acid digestion ($HCl:HNO_3$, 4:1, v/v) of the samples (McGrath & Cunliffe, 1985). In order to identify the occurrence of sorption of Zn and Pb onto macrophyte fragments (i.e. particulate or dissolved phase), an additional assay under similar condition of anaerobic decomposition assay was performed. Adsorption experiments were carried out with 100 ml of 95.3 mg Zn L$^{-1}$, 2.0 mg Zn L$^{-1}$, 0.06 mg Pb L$^{-1}$ and 6.0 mg Pb L$^{-1}$ solutions with 1.0 g of dry fragments of *H. coronarium* and *U. subquadripara* and filtered water samples from the sampling sites. Experiments were conducted under continuous shaking and contact time 12 h in the dark at 25 °C (modified from Twardowska & Kyziol, 2003). After shaking, the samples were centrifuged at 2.191 × g (30 min at room temperature) to fractionate the particulate and dissolved fraction. The glassware used in the sorption assays was also washed with an acid solution (1M $HNO_3$) to measure the Zn and Pb concentration, eventually, adsorbed onto the glassware wall. The quantification of metals (detection limit = 0.01 mg Pb L$^{-1}$ and 0.002 mg Zn L$^{-1}$) was conducted by atomic absorption spectrometry (Varian, model AA 240 FS) as describe in method SM3111B from APHA (1998).

### *Anaerobic decomposition assay*

Anaerobic microcosms were setup in glass bottles (volume 450 ml) with lids wherein 4.0 g plant dry mass were added to 400 ml of filtered water (Gimenes *et al.*, 2013) and 4 ml of microbial indigenous inoculant (based on experiments from Cunha-Santino & Bianchini Jr., 2013). According to Bianchini Jr. *et al.* (2014) based on the kinetics of oxygen consumption during macrophytes decomposition the anaerobic condition was achieved on maximum of 2 h after the introduction of plant fragments in the water. To ensure the anaerobic conditions, after being prepared, the chambers used in the anaerobic processes were closed, being opened only on the sampling days.

The treatments of microcosms were established (Table 1) considering: (i) two thermal conditions (representing the low and high mean temperatures from Savanna from the sampling region), (ii) four treatments enriched with Zn and Pb in two concentrations each (with different order of magnitude) and (iii) two control treatments with $KNO_3$ addition and only with water from the Espraiado and Fazzari reservoirs. For each treatment (Table 1) series (sampling days = 8), the microcosms (n = 3) were incubated in controlled temperature (15 and 25°C, darkness). These conditions correspond to the mean winter and summer temperatures registered in these reservoirs (Dornfeld & Fonseca-Gessner, 2005; Fusari & Fonseca-Gessner, 2006). During the experiment, the microcosms were sampled to test the presence of Zn and Pb response on decomposition. Nitrate salts of metals were added to the microcosms (the final nominal concentrations as presented in Table 1). The control treatment (C1) was also set without metal addition. An additional control treatment (C2) was prepared with nitrate potassium salt with the same concentration of the higher Zn nitrate concentration (Zn1) among all treatments (Table 1). Mean value of nitrate in the Fazzari and Espraido reservoirs was 0.45 mg $L^{-1}$ (Saltarelli *et al.*, 2018). The use of nitrate salts in bioassays as a metal source was based on studies concerning with the influence of Zn and Pb on heterotrophic microbial metabolism (Sobolev & Begonia, 2008; Feng *et al.*, 2018). In addition, the nitrate was used to avoid nitrogen limitation during decomposition. $KNO_3$ was considered as a control treatment, once is not a toxic element and present potassium and nitrogen as a nutrient source. Our motivation to use the Zn and Pb concentrations in decomposition (Table 1) were focused on higher concentrations of these metals often found in mine waste (Schaider *et al.*, 2007) and industrial effluents (Lin & Harichund, 2011). In Doce River (Mariana, MG, Brazil) the concentration of Pb varied from 0.013 to 0.097 mg $L^{-1}$ (Carvalho et al., 2017). The lower concentrations of Zn and Pb was adopted to represent the same magnitude order of Class 3 (Conama Resolution nº 357/2005: Pb = 0.033 mg $L^{-1}$ and Zn = 5 mg $L^{-1}$). The higher concentration was adopted to verify an extreme event of contamination on aquatic decomposition.

In each sampling day (1, 3, 5, 10, 25, 40, 60 and 100 days) the dissolved fraction of decomposition chambers (n = 3 for each treatment; totalizing 24 microcosms per treatment - Table 1) was isolated from particulate fraction by filtering both fractions through acetate cellulose 0.45 µm pore diameter membrane. The dissolved organic carbon fraction of each chamber was filtered. Dissolved organic carbon (DOC) was quantified by Shimadzu carbon analyzer (TOC-L, combustion and infrared detection method). Remaining particulate detritus was oven-dried (40°C) until constant mass. Subsamples (1.0 g) of particulate detritus of each chamber were ground in a Wiley mill and calcined (for 2 hours at 550°C) to obtain organic matter content. The particulate organic carbon (POC) was calculated by multiplication of the particulate remaining material (ash free basis) by 0.47 (Wetzel, 2001).

### *Data analysis*

POC decay of both macrophytes was parameterized by fitting experimental data to a kinetic model that considers detritus heterogeneity showing a labile/soluble fraction ($POC_{LS}$) and a refractory fraction ($POC_R$). Both fractions decay were represented by Equation 1, which considers a fast and slow mass loss of $POC_{LS}$ and $POC_R$, respectively (Lousier & Parkinson, 1976). The temporal changes of DOC were represented by Equation 2 (Bianchini Jr. & Cunha-Santino, 2011).

$$POC = POC_{LS} \times e^{-k_T \times t} + POC_R \times e^{-k_4 \times t} \qquad \text{(Eq. 1)}$$

$$DOC = \frac{k_2}{k_T} \times POC_{LS} \times \frac{k_T}{k_3 - k_T} \times \left(e^{-k_T \times t} - e^{-k_3 \times t}\right) \qquad \text{(Eq. 2)}$$

where: POC: particulate organic carbon (%); $POC_{LS}$: labile soluble particulate fraction of POC (%); $POC_R$: refractory fraction of POC (%); $k_T$: $POC_{LS}$ mass loss coefficient (by leaching and mineralization processes); $k_T = k_1 + k_2$ ($k_1$ = mineralization coefficient and $k_2$ = leaching coefficient) $day^{-1}$; $k_3$: DOC mass loss coefficient (by mineralization processes), $day^{-1}$; $k_4$: $POC_R$ mass loss coefficient (by mineralization processes), $day^{-1}$.

Table 1. Description of the treatments conditions in relation to temperature and metal concentration.

| Treatments | Conditions |
|---|---|
| Zn1 (15°C) | 95.3 mg Zn $L^{-1}$ |
| Zn1 (25°C) | |
| Zn2 (15°C) | 2.0 mg Zn $L^{-1}$ |
| Zn2 (25°C) | |
| Pb1 (15°C) | 6.0 mg Pb $L^{-1}$ |
| Pb1 (25°C) | |
| Pb2 (15°C) | 0.06 mg Pb $L^{-1}$ |
| Pb2 (25°C) | |
| C1 (15°C) | Control 1 |
| C1 (25°C) | |
| C2 (15°C) | Control 2 |
| C2 (25°C) | (nitrate addition) |

As consequence, the kinetic model considers that $POC_{LS}$ mass loss occur by two routes: (i) direct oxidation of $POC_L$ ($IN_1$ route) and (ii) DOC formation ($k_2$) followed by DOC mineralization ($k_3$) ($IN_2$ route). $POC_R$ mineralization is related to $k_4$ coefficient (route $IN_3$). By this way, the mineralization pathways (inorganic or mineralized carbon production - MC) are depicted by the sum of $IN_1$, $IN_2$ and $IN_3$ routes (Equations 3, 4, and 5).

$$IN_1 = POC_L \times (1 - e^{-k_T \times t}) \quad \text{(Eq. 3)}$$

$$IN_2 = POC_S \times \left(1 + \frac{k_3}{k_T - k_3} \times e^{-k_T \times t} + \frac{k_T}{k_3 - k_T} \times e^{-k_T \times t}\right) \quad \text{(Eq. 4)}$$

$$IN_3 = POC_R \times (1 - e^{-k_4 \times t}) \quad \text{(Eq. 5)}$$

The temporal changes in POC and DOC from incubations were used to achieve the model parameters. The parameterization was done by no-linear regressions, using the Levenberg-Marquardt iterative algorithm (Press *et al.*, 1993). Half-life of decay processes (t½) were calculated by Equation 6.

$$t_{½} = \frac{\ln(0.5)}{-k} \quad \text{(Eq. 6)}$$

where: k: reaction rate constant (i.e. $k_T$, $k_3$, $k_4$), $day^{-1}$.

Temperature effect on POC decay under 15 and 25°C was evaluated by $Q_{10}$ coefficientbased on Arrhenius equation approach (Equation 7).

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad \text{(Eq. 7)}$$

where: $K_1$ = reaction rate at temperature $T_1$; $K_2$ = reaction rate at temperature $T_2$ and $Q_{10}$ = ratio of reaction rates at 10 ºC temperature increments. The overall $Q_{10}$ values were determined by linear regressions among the carbon mineralized yields at 15 and 25 ºC.

The POC decay values from kinetic fittings in each treatment were submitted to the Kolmogorov-Smirnov test to check normality of the distribution and to Bartlett test to verify homoscedasticity. As these conditions were fulfilled, the POC decay of each treatment was submitted to the parametric ANOVA-repeated tests. Whenever significant differences among treatments and species were found, post hoc Tukey test was carried out to identify differences among treatments. Differences were considered significant where $p < 0.05$.

## RESULTS

The background values of metals in the *H. coronarium* and *U. subquadripara* tissues were 0.008 mg Pb $g^{-1}$, 0.07 mg Zn $g^{-1}$ and 0.0105 mg Pb $g^{-1}$, 0.05 mg Zn $g^{-1}$, respectively. The concentrations of these metals in the water were 0.002 mg Pb $L^{-1}$ and 0.02 mg Zn $L^{-1}$ (Fazzari Reservoir), and 0.001 mg Pb $L^{-1}$ and 0.03 mg Zn $L^{-1}$ (Espraiado Reservoir). Metal adsorption in the presence of Zn and Pb (Figure 1) indicated that the metals exhibited different yields adsorption onto dissolved and particulate phases of *H. coronarium* and *U. subquadripara* incubations. Adsorption of Zn and Pb was preferentially binding onto particulate than dissolved fraction. In the Zn treatments, the particulate phase (i.e. POC) adsorption varied from 91.4 (Zn2 - *U. subquadripara*) to 97.3% (Zn1 - *H. coronarium*). In the Pb treatments these values ranged from 90.0 (Pb2 - *U. subquadripara*) to 97.9% (Pb1 - *H. coronarium*). The yields in the dissolved phase varied from 1.9 to 8.4% for Zn and 1.5 to 9.5% for Pb. The mean value of adsorption onto the glassware wall was 1.1% for Zn and 0.6% for Pb (Figure 1).

The temporal variation of POC decay, DOC formation and MC evolution during *H. coronarium* and *U. subquadripara* decomposition for C1 and C2 treatments was presented in Figure 2. The MC from *H. coronarium* degradation from these treatments was intermittent and tenuous. For C2, the mineralization was late (from 25th day at 25 °C and 40th at 15 °C). Contrasting C1 and C2 kinetic parameters (Table 2: *H. coronarium* and Table 3: *U. subquadripara*), the $k_4$ was higher in C2 in both macrophytes detritus and temperature, except for *H. coronarium* at 15 °C ($k_4 = 0.0017\ day^{-1}$).

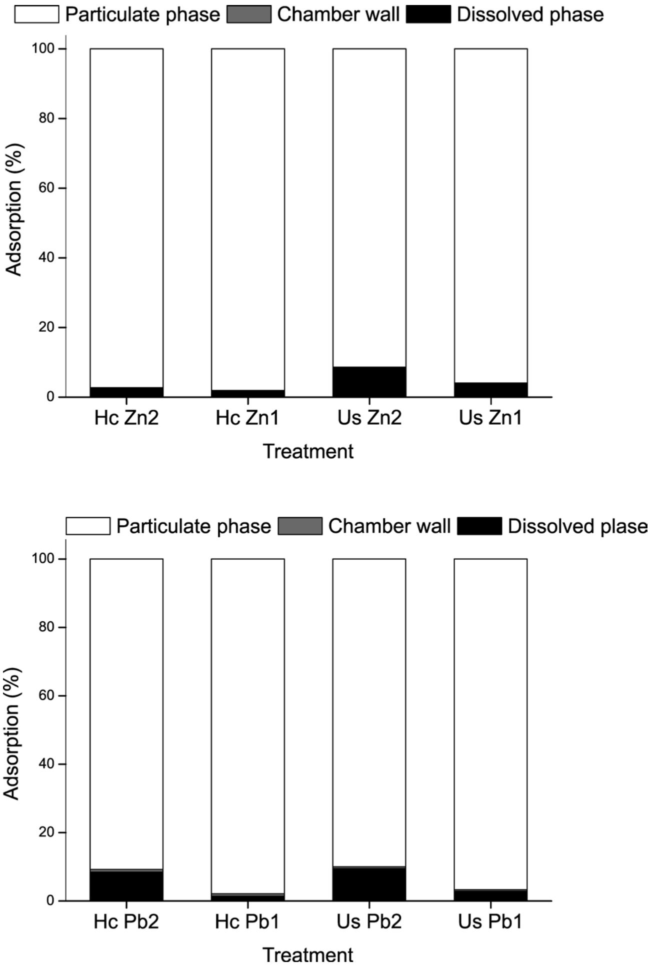


Fig. 1. Yields of adsorption of Zn and Pb onto the organic detritus of the decomposition chamber (i.e. dissolved carbon and particulate carbon) and glassware wall (Hc = *Hedychium coronarium* and Us = *Urochloa subquadripara*).

During the decomposition period (Figures 3 and 4) the detritus of *H. coronarium* and *U. subquadripara* at both temperatures, evolved through three carbon pathways: (i) POC, (ii) DOC and (iii) mineralized carbon (MC = sum of $IN_1$, $IN_2$ and $IN_3$). The formation of two fractions during decomposition indicates the heterogeneity of detritus, a labile/soluble ($POC_{LS}$) and refractory carbon content ($POC_R$). Specific in the decomposition of *H. coronarium*, the MC was intermittent, representing a residual process, mainly in the Zn treatments. The higher MC yields were observed in the Pb treatments principally at 25 °C (Figure 3). The increase of the yield of the $POC_{LS}$ was observed, in all treatment, from the first day of decomposition. The yield of $POC_{LS}$ obtained from kinetic model varied from 7.8 to 12.7% for *H. coronarium* (Table 2) and from 12.5 to 25.0% for *U. subquadripara* detritus (Table 3).

Regardless the treatments (i.e. temperature and plant type) the predominant form of carbon remains as particulate detritus (*H. coronarium* $POC_R$ variation: 70.3 to 98.9% and *U. subquadripara* $POC_R$ variation: 60.9 to 79.2%). The percentage of the $POC_R$ yield is complementary to the $POC_{LS}$ yield. Considering the temperature, during *H. coronarium* decay at 25 °C a higher yield of DOC was observed (Figure 2). This same pattern was also observed during the *U. subquadripara* detritus decay (Figure 3).

In the *H. coronarium* decomposition at 15 °C, all treatments exhibited a POC decrease since the 1st day of incubation, concomitant to the increase of the DOC content, until the end of experiment. Among treatments, Zn1 presented the lower DOC formation during *H. coronarium* decomposition (Figure 3). The absence increasing in DOC formation found in the Zn1 treatment was not observed for the *U. subquadripara* decay (Figure 4). Significant difference ($p < 0.01$) was obtained in the Zn1 condition in comparison to all treatments in both temperatures, except for the Zn1 at 25 °C ($p > 0.05$). In the C2 (at 15 °C), the decomposition of both macrophytes presented significant difference from treatments submitted to 25 °C ($p < 0.01$) except for the treatment Zn2. The Zn2 (15 °C) was distinct from the two controls (C1 and C2) treatment processed under 25 °C for both *H. coronarium* and *U. subquadripara*.

At 25 °C, in the decomposition of the *H. coronarium,* the same differences among treatments as verified at 15 °C were observed (Figure 2). It is important to highlight the noticeable effect of the highest Zn concentration (Zn1) in treatments at 15 and 25 °C that do not allowed $k_T$ and $k_4$ evaluations. The Zn1 condition inhibited DOC temporal formation and the POC decay. A surprisingly aspect regarding to the decomposition of *H. coronarium* was the lack of MC formation under Zn1 treatments at 15 °C and 25 °C. Concerning to the increasing temperature in the decomposition of the *H. coronarium*, significant different were obtained from decay patterns from Zn1 treatment at 15 °C and 25 °C in relation to all others treatments ($p < 0.001$). Among all treatments, Zn1 degradation was similar to each other ($p > 0.05$). Regarding to the controls (C1 at 25 °C) was different ($p < 0.01$) for C2 (15 °C).

POC temporal variation (Figure 2) during the decomposition of *U. subquadripara* from both controls was distinct between temperatures ($p < 0.01$). At 15 °C, the C1 and C2 showed similar decay yields than Zn1, Pb1 and Pb2

Table 2. Kinetics parameters from *Hedychium coronarium* decay under 15 and 25 °C and metal (Zn and Pb) treatments.Where: $POC_{LS}$: labile soluble particulate fraction of POC (POC: particulate organic carbon; %); $POC_R$: refractory fraction of POC (%); $k_T$: $POC_{LS}$ mass loss coefficient (by leaching and mineralization processes), $k_T = k_1 + k_2$ ($k_1$ = mineralization coefficient and $k_2$ = leaching coefficient) $day^{-1}$; $k_3$: DOC mass loss coefficient ($COD \approx COP_{LS}$ when $COPL \neq 0$), $day^{-1}$; $k_4$: $POC_R$ mass loss coefficient (by mineralization processes), $day^{-1}$; $Q_{10}$ = ratio of reaction rates at 10 °C temperature increment.

| | Parameters | Treatments | | | | | |
|---|---|---|---|---|---|---|---|
| | | C1 | C2 | Zn1 | Zn2 | Pb1 | Pb2 |
| 15°C | $COP_{LS}$ (%) | 9.1 | 12.5 | 8.9 | 7.8 | 10.5 | 8.2 |
| | $k_T$ ($day^{-1}$) | 1.5 | 1.5 | - | 0.5 | 0.9 | 2.9 |
| | $t_{½LS}$ (day) | 0.5 | 0.5 | - | 1.4 | 0.8 | 0.2 |
| | $k_3$ ($day^{-1}$) | 0 | 0 | 0 | 0 | 0 | 0 |
| | $COP_R$ (%) | 90.9 | 87.5 | 91.1 | 92.2 | 89.5 | 91.8 |
| | $k_4$ ($day^{-1}$) | 0.0019 | 0.0017 | 0 | 0.0014 | 0.0012 | 0.0016 |
| | $r^2$ | 0.97 | 0.88 | - | 0.98 | 0.98 | 0.94 |
| | $t_{½R}$ (day) | 365 | 408 | - | 495 | 578 | 433 |
| 25°C | $COP_{LS}$ (%) | 12.3 | 12.3 | 12.3 | 12.3 | 12.7 | 12.3 |
| | $k_T$ ($day^{-1}$) | 2.4 | 1.1 | - | 0.15 | 0.34 | 0.35 |
| | $t_{½LS}$ (day) | 0.3 | 0.6 | - | 4.6 | 2.0 | 2.0 |
| | $k_3$ ($day^{-1}$) | 0 | 0 | 0.12 | 0 | 0 | 0 |
| | $COP_R$ (%) | 87.7 | 87.7 | 87.7 | 87.7 | 87.3 | 87.7 |
| | $k_4$ ($day^{-1}$) | 0.0021 | 0.0028 | 0 | 0.0014 | 0.0019 | 0.0024 |
| | $r^2$ | 0.97 | 0.87 | - | 0.95 | 0.98 | 0.94 |
| | $t_{½R}$ (day) | 330 | 248 | - | 495 | 365 | 288 |
| | $Q_{10}$ | 1.23 | 1.75 | 0.95 | 1.26 | 1.27 | 1.38 |

Table 3. Kinetics parameters from *Urochloa subquadripara* from decay15 and 25 °C and metal (Zn and Pb) treatments. Where: $POC_{LS}$: labile soluble particulate fraction of POC (POC: particulate organic carbon; %); $POC_R$: refractory fraction of POC (%); $k_T$: $POC_{LS}$ mass loss coefficient (by leaching and mineralization processes), $k_T = k_1 + k_2$ ($k_1$ = mineralization coefficient and $k_2$ = leaching coefficient) $day^{-1}$; $k_3$: DOC mass loss coefficient (COD ≈ $COP_{LS}$ when COPL ≠ 0), $day^{-1}$; $k_4$: $POC_R$ mass loss coefficient (by mineralization processes), $day^{-1}$; $Q_{10}$ = ratio of reaction rates at 10 °C temperature increment.

| | Parameters | Treatments | | | | | |
|---|---|---|---|---|---|---|---|
| | | C1 | C2 | Zn1 | Zn2 | Pb1 | Pb2 |
| 15°C | $COP_{LS}$ (%) | 15.1 | 12.6 | 14.8 | 14.5 | 12.5 | 15.0 |
| | $k_T$ ($day^{-1}$) | 1.4 | 1.5 | 0.7 | 1.6 | 1.9 | 0.1 |
| | $t_{½LS}$ (day) | 0.5 | 0.5 | 1.0 | 0.4 | 0.4 | 6.9 |
| | $k_3$ ($day^{-1}$) | 0 | 0 | 0 | 0 | 0 | 0 |
| | $COP_R$ (%) | 84.9 | 87.4 | 85.2 | 85.5 | 87.5 | 85.0 |
| | $k_4$ ($day^{-1}$) | 0.0013 | 0.0017 | 0.0010 | 0.0016 | 0.0013 | 0.0007 |
| | $r^2$ | 0.97 | 0.94 | 0.93 | 0.92 | 0.99 | 0.92 |
| | $t_{½R}$ (day) | 533 | 408 | 685 | 435 | 544 | 990 |
| 25°C | $COP_{LS}$ (%) | 15.0 | 13.0 | 15.3 | 25.0 | 22.0 | 18.0 |
| | $k_T$ ($day^{-1}$) | 0.2 | 1.5 | 0.7 | 0.4 | 0.4 | 0.2 |
| | $t_{½LS}$ (day) | 3.5 | 0.5 | 1.0 | 1.7 | 1.7 | 3.5 |
| | $k_3$ ($day^{-1}$) | 0 | 0.003 | 0 | 0 | 0 | 0.005 |
| | $COP_R$ (%) | 85.0 | 87.0 | 84.7 | 75.0 | 78.0 | 82.0 |
| | $k_4$ ($day^{-1}$) | 0.0041 | 0.0054 | 0.0026 | 0.0022 | 0.0017 | 0.0019 |
| | $r^2$ | 0.84 | 0.76 | 0.91 | 0.96 | 0.90 | 0.99 |
| | $t_{½R}$ (day) | 169 | 128 | 267 | 315 | 408 | 365 |
| | $Q_{10}$ | 1.48 | 1.59 | 1.26 | 1.50 | 1.49 | 1.30 |

($p < 0.05$). In the Zn2 treatment, the carbon loss in the lower temperature was different from Pb1 and Pb2, and from Pb2 (25 °C). At 25 °C, the POC decay of controls (C1 and C2) showed no significant differences only with Zn1 and Pb2 ($p < 0.05$). The determination coefficients ($r^2$) from kinetic fittings indicated a proper adherence of experimental data to the proposed mathematical model (variation from 0.76 to 0.99; Tables 2 and 3).In the decomposition of the *H. coronarium*, the raise in the temperature induced a positive effect in the $k_4$ being, on average, 1.36 times higher at 25 °C. This relation was confirmed by $Q_{10}$ (Table 2) that showed an average value of 1.31 when considering all treatments. The influence of temperature on the $k_4$ in the decomposition of *U. subquadripara* was higher (2.36 times between 15 and 25 °C) than observed for *H. coronarium*. The $Q_{10}$ values observed in the decomposition of the *U. subquadripara* varied from 1.26 to 1.59 (Tables 2 and 3). For both plants, the $k_T$ was not influenced by the thermal regime. The $k_T$ determined by the kinetic fittings was 3 orders of magnitude higher than $k_4$ (Tables 2 and 3).

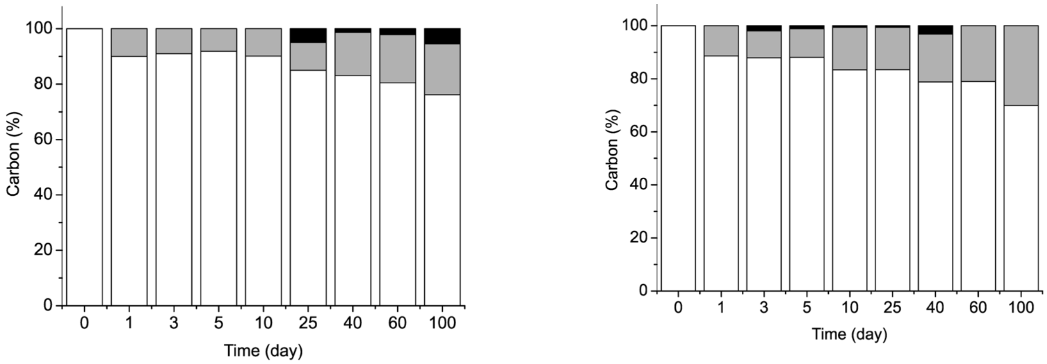
In the DOC mineralization (represented by $k_3$; Equation 4) for both plants detritus, we observed a null $k_3$; except for *U. subquadripara* in the C2 (0.003 $day^{-1}$; $t_{½}$ = 231 days), Pb2 treatments at 25 °C (0.005 $day^{-1}$; $t_{½}$ = 138 days) (Table 3) and the Zn1 treatment in the *H. coronarium* DOC mineralization at 25 °C (0.12 $day^{-1}$; $t_{½}$ = 6 days). The pattern described by the null $k_3$ was the temporal increasing of COD concentrations throughout the experimental period therefore, the COD decay was not observed.

## DISCUSSION

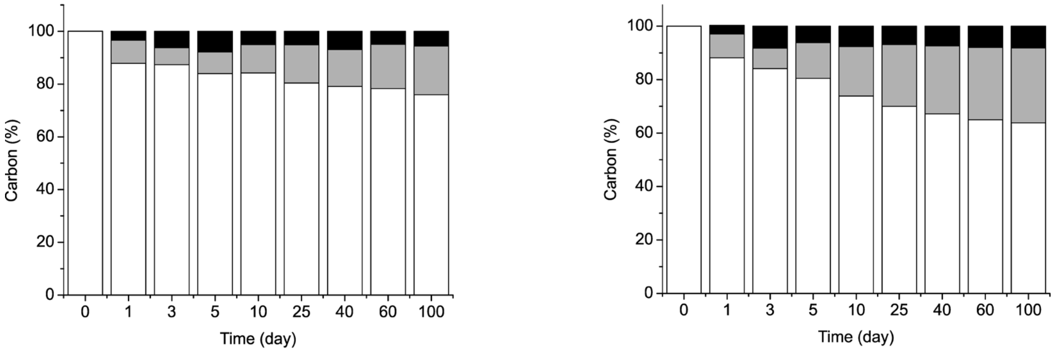
The biosorption was the predominant process in the POC of the decomposition chamber, meaning that the particulate detritus was subject to interplay between Zn and Pb and heterotrophic processing ability of attached microorganisms and free-living bacteria in the freshwater carbon cycling. Dried biomass and detritus of macrophytes has been used successfully as natural biosorbents for metals, as Zn and Pb (Schneider & Rubio, 1999; Miretzky *et al.*, 2006) indicating that plant detritus is subject to metal adsorption. In this context, the adsorption of metals in macrophytes detritus during decomposition was observed in field experiments (Azeez & Prusty, 2008).

Regardless of plant type, temperature incubation and treatments with Zn or Pb, decomposition of detritus showed two distinct phases: (i) a fast decay in the initial phase of degradation and (ii) a posterior slower decay. This biphasic decay pattern is often attributed to the heterogeneity of detritus that displayed a labile and/or soluble and a refractory fraction (Mancinelli *et al.*, 2005). The detritus behaved as comprising two compartments, one labile that decreased quickly and the other (i.e. refractory) that decreased 10-20 times more slowly (Gillon *et al.*, 1994).

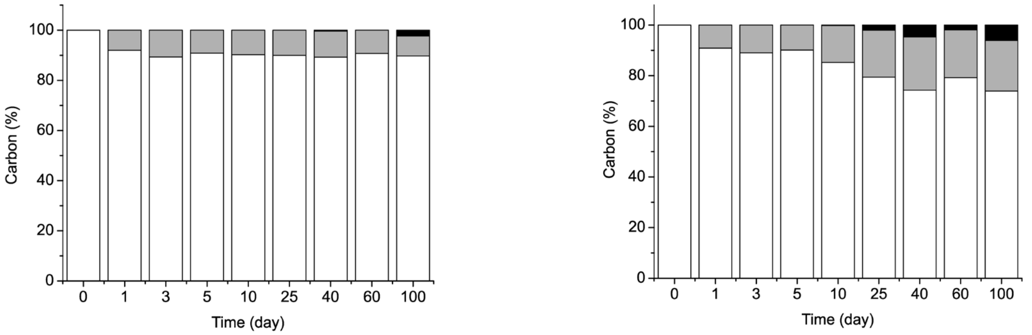
The $POC_{LS}$ decay represents the first step of carbon dynamics in the particulate detritus once this fraction originated the DOC in the early phase of decomposition. This process is due to the leaching of hydrosoluble carbonaceous



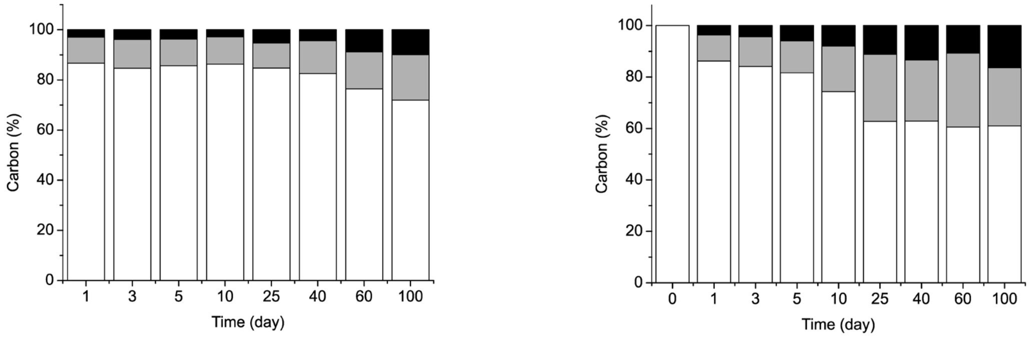
C1 15ºC – *H. coronarium* C1 25ºC - *H. coronarium*



C1 15ºC – *U. subquadripara* C1 25ºC - *U. subquadripara*

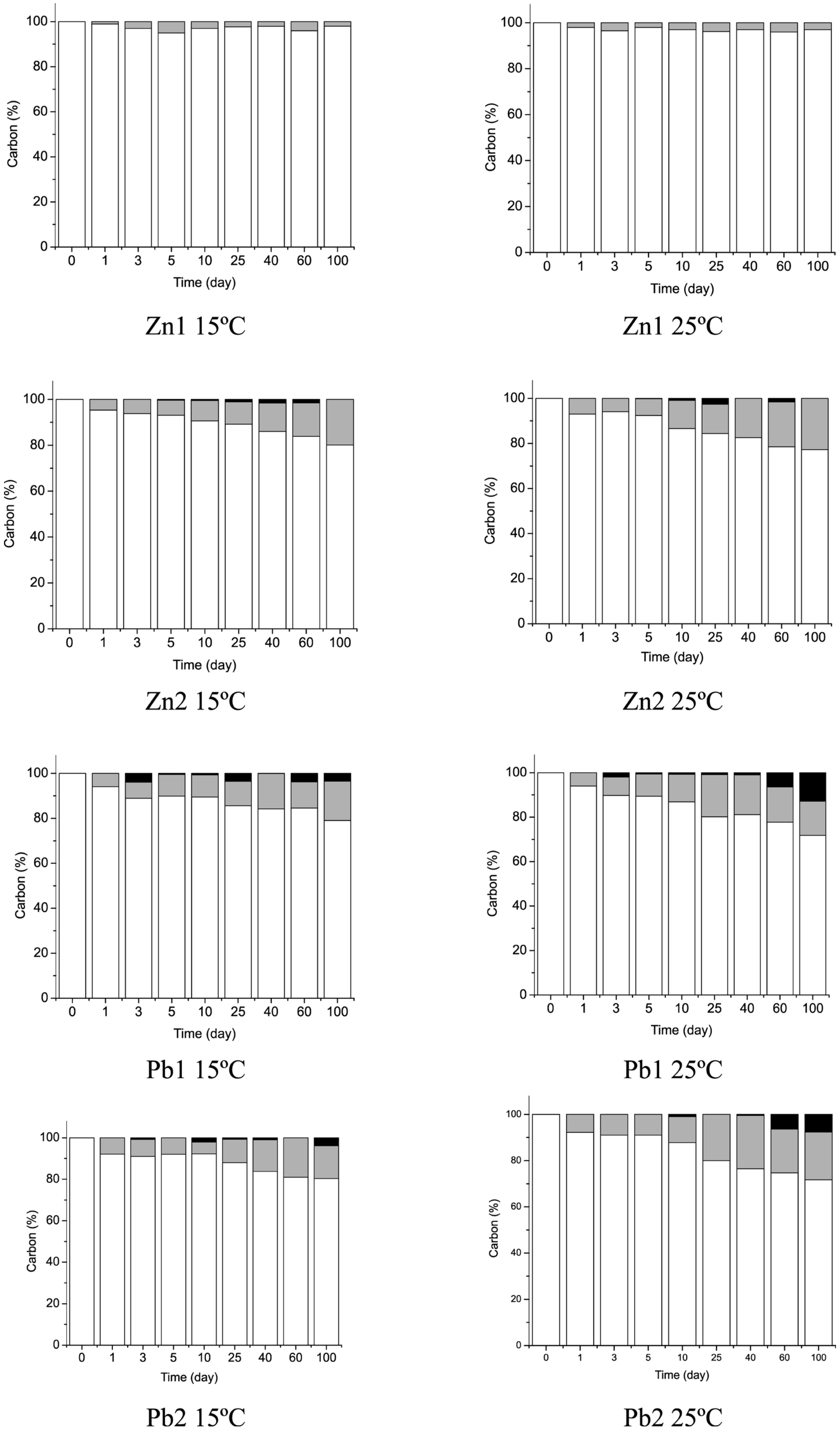


C2 15ºC - *H. coronarium* C2 25ºC - *H. coronarium*



C2 15ºC - *U. subquadripara* C2 25ºC - *U. subquadripara*

Fig. 2. Carbon balance from anaerobic decomposition of *Hedychium coronarium* and *Urochloa subquadripara*at 15°C and 25°C in C1 and C2 treatments (gray = DOC, white = POC and black = MC).



Zn1 15ºC

Zn1 25ºC

Zn2 15ºC

Zn2 25ºC

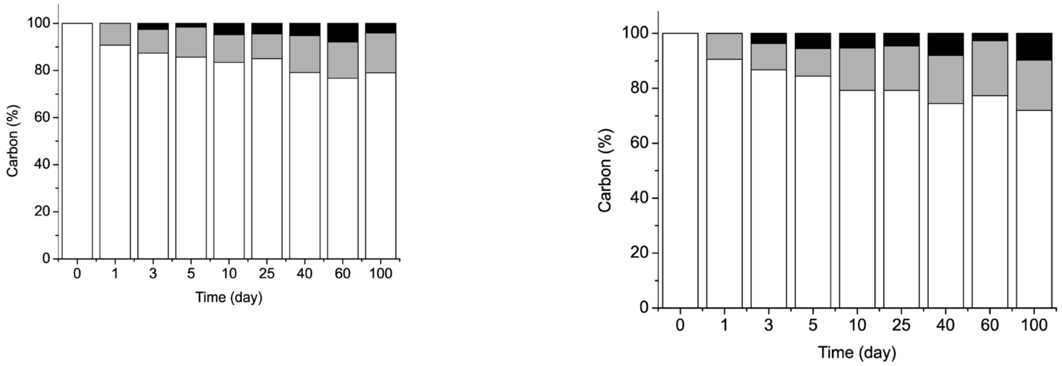
Pb1 15ºC

Pb1 25ºC

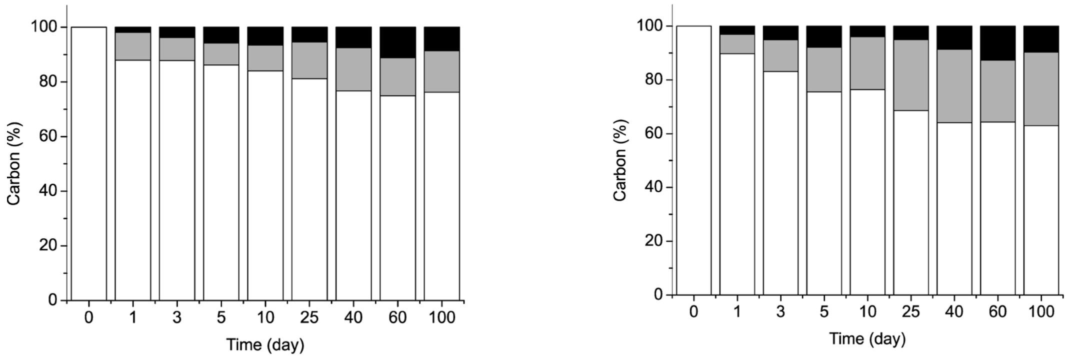
Pb2 15ºC

Pb2 25ºC

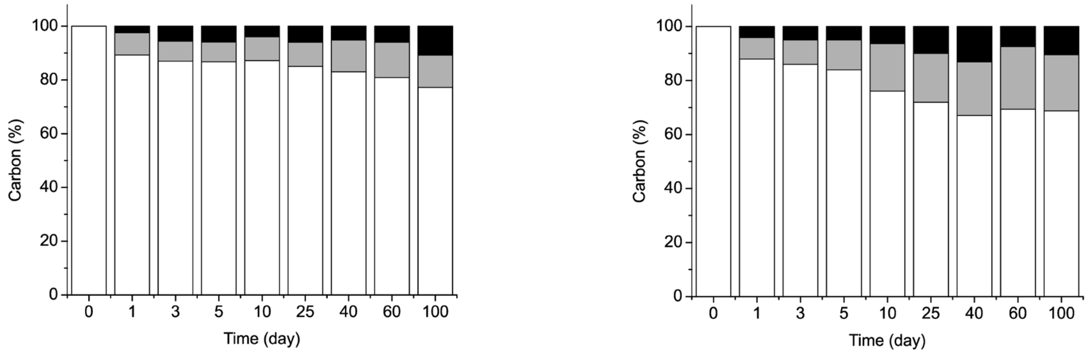
Fig.3. Carbon balance from anaerobic decomposition of *Hedychium coronarium* at 15 °C and 25 °C submitted to Pb and Zn treatments (gray = DOC, white = POC and black = MC).



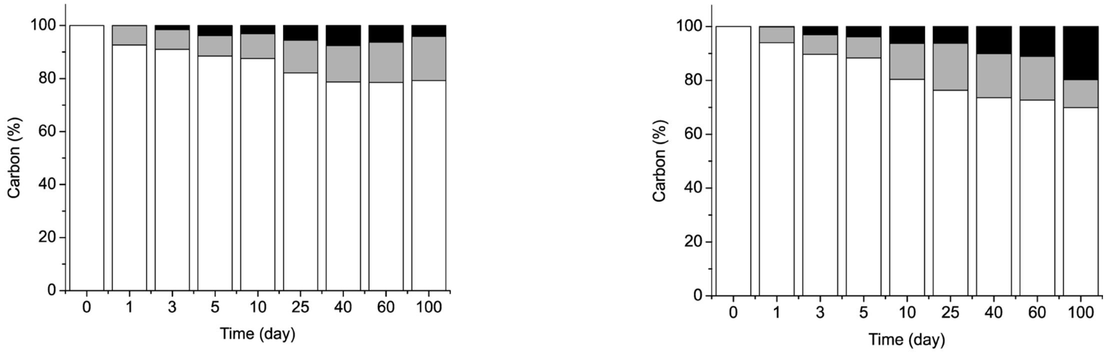
Zn1 15ºC Zn1 25ºC



Zn2 15ºC Zn2 25ºC



Pb1 15ºC Pb1 25ºC



Pb2 15ºC Pb2 25ºC

Fig.4. Carbon balance from decomposition of *Urochloa subquadripara* at 15 °C and 25 °C submitted to Pb and Zn treatments (black = MC, gray = DOC and white = POC).

compounds from protoplasmatic content of plant detritus that is primarily composed of polysaccharides, carboxylic acids, amino acids, proteins and phenolic acids (Liu *et al.*, 2016). Other elements are also released during leaching process and P compounds seemed to be the most soluble when compared to the C and N compounds (Gillon *et al.*, 1994). The $POC_{LS}$ is often faster degraded by heterotrophic organisms, however, our results indicated that the presence of Zn1 when compare to the control experiment decreased the decay rate and metabolism of the compounds presented in this fraction.

The concentration of DOC leachated from the *H. coronarium* detritus showed an increasing pattern over the experimental period. Decomposition studies usually reports an initial increase of DOC followed by a decrease tendency concomitant with MC formation (Bottino *et al.*, 2013). The DOC increasing concentrations trend suggests a refractory nature of DOC (Cunha-Santino *et al.*, 2010) or that this fraction was not rather used by heterotrophic microorganisms and owning of the metal presence. In fact, the DOC uptake was suppressed in all treatment during decomposition of *H. coronarium*. The increase in DOC concentration along experimental period associated with the lack of MC unable to performance the fitting using Equation 2. Probably, the negligible MC evolved in the *H. coronarium* decay was derived from chemical oxidation. In addition, during the decomposition of *H. coronarium*, the antimicrobial substance from this plant (Aziz *et al.*, 2009) also contributed to the lack of mineralization of the dissolved and particulate detritus inhibiting the microbial activity. The antimicrobial activity in the rhizome substances (e.g. trans-meta-mentha-2,8-diene, linalool, α-terpineol, terpin-4-ol; α-pirene, γ-terpinene and camphene) from *H. coronarium* was previously reported (Joshi *et al.*, 2008). Different from *H. coronarium* decomposition, the *U. subquadripara* decay showed the microbial-mediated mineralization represented mainly by the MC evolved from mineralization of both fractions, the $POC_{LS}$ and $POC_R$.

The potassium ions are an essential macronutrient for organisms and in bacteria, this element act as an activator of intracellular enzymes (Epstein, 2003). K is a nutritional requirement acting as cofactor in enzymatic activities, including those involved in protein synthesis (Atlas, 1997). The presence of K, as a non-limiting element for microbial metabolism was observed in the mineralization of $POC_R$ ($k_4$) of C2 when compared to C1.

According to our results, a question arose, i.e. why Zn1 treatment in the decomposition of *H. coronarium* and *U. subquadripara* displayed different trend under both temperatures incubation? Although bacterial resistance mechanics against of Zn is achieved by immobilization, turning this metal less toxic to metabolism stress (Ahemad & Malik, 2012); distinct mechanisms of metal microbial toxicity are listed (Ahemad, 2012): protein denaturation (Pb), inhibition of cell division (Pb), cell membrane disruption (Pb and Zn), inhibitory of enzyme activity (Zn and Pb), DNA damage (Pb) and transcription inhibition (Pb). Considering that hydrolysis is a rate limiting step in anaerobic degradation of organic polymers and this process is carried out by hydrolases, an extra cellular enzyme (Vavilin *et al.*, 2008), a possible explanation of Zn1 inhibition of decay in *H. coronarium* was the inhibitory of hydrolases activity.

Another point to consider in the carbon cycling, since it is a biological mediated process, is the metabolism and number of microbial communities during the degradation process. Considering that the number of heterotrophic bacteria, the presence of Zn up to 0.7 mg $L^{-1}$ in freshwater environment presented positive relationships between bacterial numbers and their hydrolytic enzyme activities (Rasmussen & Olapade, 2016). An inhibitory effect on glucose mineralization rates as response by microbial activities in water samples was observed as increasing concentrations of Zn (Romero *et al.*, 1999). The presence of Pb (0.62 g $L^{-1}$) inhibits the β-glucosidase activity of the heterotrophic component in freshwater biofilm (Fechner *et al.*, 2010).

The effect of the treatments Zn2, Pb1 and Pb2 in the *U. subquadripara* and *H. coronarium* decay was not evident as Zn1 treatment. Owing the soil composition (i.e. red-yellow latosol), the amount of Zn in the sediments of the Espraiado (3.95 mg $kg^{-1}$) and Fazzari (6.75 mg $kg^{-1}$) reservoirs was high (Corbi *et al.*, 2008), indicating that this metal, depending on environment conditions, can represents a potential regulator *in situ* on the decay of organic matter of these plants, also in non-perturbed conditions.

As observed for the *H. coronarium* decomposition, the temperature showed a positive correlation with *U. subquadripara* POC decay, representing by $Q_{10}$ values. The values of $Q_{10}$ obtained in the present study indicated a response to the temperature increase related to the microbial metabolic process. This correlation is often found in decomposition studies. $Q_{10}$ values found have presented similar values of that found by Bottino *et al.* (2013). Temperature affects microorganism metabolic rates by increasing enzymatic reactions velocity (Davidson & Janssens, 2006) until a certain limit, from which rates begins to decrease due enzyme denaturation. In the range from 5 to 35°C, the $Q_{10}$ value of 2 is often an adequate value when considering the regulation of temperature on the decomposition as an ecosystem response (Kätterer *et al.*, 1998). The $Q_{10}$ showed clearly that the increase in temperature affect the efficiency of organic matter turnover from *H. coronarium* and *U. subquadripara* in anaerobic conditions.

Considering the premise of this study that Pb and Zn could act as rate-regulating agents on the anaerobic decomposition of *H. coronarium* and *U. subquadripara*, our results showed that in the decomposition these species, only the higher concentrations of Zn (Zn1 Treatment) inhibited decomposition of POC. The *H. coronarium* also presented a surprise response in the decay of detritus once a tenuous MC fraction was observed during our experimental time. In literature, it is well known the antimicrobial effect of this plant (e.g. Joshi *et al.*, 2008). From a systemic point of view, after senescence of this plant, the accumulation and the very slow degradation of detritus the carbon turnover represent a longtime process, acting as a sink of carbon in the biomes invaded by this specie.

Anoxic conditions occur throughout the lentic environment and processing of organic matter, derived from aquatic macrophytes, in these aquatic systems contributes substantially to the global cycling of carbon. Understanding the potential for the degradation of POC is therefore essential to establish the link between the input of macrophytes detritus and the turnover of the carbon is aquatic systems. The high concentration of metals in the sediments of the lentic systems subject to human action can act over the metabolism microbial community (e.g. mortality rates and decrease of the enzymes production) that mediated decomposition process. Metals can influence the yield of carbon mass balance as temperature and other driving forces, as discussed by Queiroz *et al.* (2018).

The ecological approach of the decomposition as a biologically mediated process considering the relationships of the metal interaction as an extrinsic controlling factor with the detritus processing as a systemic model. The modeling of decomposition process related to carbon cycling in aquatic environments is important for understanding the heterotrophic capacity of detritus processing in metal-affected environments. As a systemic potential ecological consequence, the reduction on the decay coefficient ($k_4$) provides the organic matter accumulation in sediments establishing the reducing conditions. From an ecosystem view, the non-mineralization occurrence, in the *H. coronarium* decomposition will provide the enrichment of a stable organic matter on the sediments of the littoral zone of this environment, thus the detritus act as a sink of carbon to the long-term dynamic of organic matter. Our results highlight the use of mathematical models to predict organic matter turnover under an ecosystem subject to metal contamination.

## CONCLUSIONS

The higher concentrations of Zn (Zn1 Treatment) inhibited or nullified decomposition of $POC_R$ acting as rate-regulating agents on the anaerobic decomposition of *H. coronarium* and *U. subquadripara*. Temperature increasing enhanced the decomposition rates ($k_4$) of $POC_R$ for both macrophytes; during the decomposition of the *H. coronarium* and *U. subquadripara*, the decay was on average, 1.36 and 2.36 times higher at 25°C, respectively.

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